cryo-transmission electron microscopy. We visualised the intermediate initial bilayer contacts and stalk formation, followed by pore development, pore evolution into 2D hexagonally packed lattices, and finally creation of 3D bicontinuous cubic structures (3).
In a biological context, the experimental corroboration of transitional lipid self-assembly structures furthers the understanding of organelle morphogenesis and maturation.
The ability to manipulate intermediate structures in nanoparticulate dispersions of self-assembled structures may provide a unique system for encapsulation and controlled release of bioactives. The capability to control intermediate transformations may also permit the development of flexible growth media for applications such as in-cubo integral membrane protein crystallization or liquid crystal templating of nanostructured materials.
1.Drummond, C.J.; et al., Current Opinion in Colloid & Interface Science 1999, 4, (6), 449-456.
2. Conn, C.E.; et al., Physical Review Letters, 2006, 96, (10), 108102
3. Mulet X., et al., ACS Nano, 2009, 3 (9), 2789-2797

**1499-Pos**
**Native Pulmonary Surfactant Membranes in Mice Show Coexistence of Two Different Phases in Bilayers and Monolayers: When the Lipid Composition can Predict the Structural Phase Segregations**
**Jorge Bernardino de la Serna**, Søren Hansen, Hans K. Hannibal-Bach, Uffe Holmskov, Adam C. Simonsen, Jens Knudsen, Luis A. Bagatolli.
University of Southern Denmark, Odense, Denmark.
Pulmonary surfactant is a surface active material composed both of lipids (aprox. 90% by weight) and proteins (aprox. 10%) produced by type II pneumocyte cells in the alveoli. This tension-active material forms a unique air-liquid interface at the alveolar cell surface that reduces surface tension close to 0 mN/m and maintains lung volumes and alveolar homeostasis at the end of the expiration. There are four pulmonary surfactant proteins (SP-A, -B, -C and -D). SP-A and -D have an important role in the immunological response against pathogens. The particular lipid composition of the lung surfactant suggests that surfactant mono- and bi-layer-based structures could exhibit lateral phase segregation at physiological temperatures. This work shows that in native pulmonary surfactant membranes a close lipid compositional study is crucial to understand the structure and biophysical function of these complex mixtures. Observing Giant Unilamellar Vesicles under conventional and two-photon excitation microscopy allow us to characterize and quantify the coexistence of two fluid-like phases in the wild-type (wt) native pulmonary surfactant membranes and a gel/fluid-like segregation pattern in the Knocked-out protein D (KOD) membranes. The atomic force microscopy studies of supported Langmuir-Blodgett bilayers and monolayers at different surface pressure show the same phase pattern before the collapse surface pressure of native pulmonary surfactant (~40mN/m). Above this surface pressure different protruded structures can be observed arising from the more fluid phases. A closer look at the lipid composition reveals a higher content of saturated phospholipid species in the KOD native pulmonary surfactant membranes. This last finding explain the coexistence phenomena observed and allow us to conclude that the pulmonary surfactant segregation pattern could be predicted by an accurate lipid compositional study.

## Membrane Receptors & Signal Transduction I

**1500-Pos**

WITHDRAWN.

**1501-Pos**
**Quantitative GPCR Assay Using Time-Resolved Fret**
**Adam M. Knepp**, Thomas P. Sakmar, Thomas Huber.
www.sakmarlab.org, Rockefeller University, New York, NY, USA.
Quantitative study of membrane proteins presents considerable technical challenges. Here, we report a time-resolved fluorescence resonance energy transfer (TR-FRET) assay to quantify functional Chemokine (C-C motif) Receptor 5 (CCR5). CCR5 is a seven-transmembrane helical G protein-coupled receptor (GPCR) expressed primarily on immune cells. It is of significant interest due to its role as the co-receptor of R5-tropic HIV-1. The TR-FRET assay exploits energy transfer between a long-lived europium cryptate donor fluorophore and an appropriate acceptor. We have developed a homogeneous sandwich-type immunoassay with labeled antibodies against a conformationally-sensitive epitope on the extracellular domain of CCR5 and an engineered C-terminal 1D4-mAb epitope. The assay yields a quantitative FRET signal corresponding to the total amount receptor. To quantitate ''functional'' receptor, a labeled anti-HA (hemagglutinin) antibody against an engineered N-terminal HA epitope is used in conjunction with labeled MIP-1a, a chemokine ligand for CCR5. We are also interested in quantifying the degree of chemical labeling of unnatural amino acids incorporated into expressed GPCRs by amber codon suppression technology. The Staudinger-Bertozzi ligation links a commercially-available FLAG-phosphine reagent to p-L-azidophenylalanine. A europium-labeled anti-FLAG antibody can then be used in another version of the sandwich assay. The resulting FRET signals are directly proportional to amount of total, functional, and labeled receptor, but must be calibrated precisely to extract an absolute concentration. Calibration is accomplished by measuring binding of a fluorescent derivative of a small-molecule CCR5 antagonist. The assay is highly specific due to the long lifetime of the europium donor, and nanomolar concentrations of receptor are detectable. This GPCR assay technology can be used to optimize CCR5 reconstitution conditions and can be readily extended to other members of the chemokine receptor family.

**1502-Pos**
**Effects of Sensory Rhodopsin II Complexation with its Cognate Transducer HtrII on the Local Environment of Internal Water Molecules**
Mikkel Jensen[1], **Erica C. Saint Clair[1]**, Alan Gabel[1], Vladislav B. Bergo[1], Elena N. Spudich[2], John L. Spudich[2], Kenneth J. Rothschild[1].
[1]Boston University, Boston, MA, USA, [2]University of Texas Medical School, Houston, TX, USA.
Recent studies indicate that internal water molecules play a critical role in membrane protein function. Here we report evidence that the local environment of one or more internal water molecules in sensory rhodopsin II (SRII) is altered by interaction with its cognate transducer HtrII. The SRII-HtrII complex mediates blue-light repellent phototaxis in halophilic archaea, using a signaling pathway similar to that in bacterial chemotaxis. We studied the photocycle of SRII and a SRII-HtrII fusion complex from *Natronobacterium pharaonis* using low-temperature static and room temperature time-resolved Fourier transform infrared (FTIR) difference spectroscopy. When cooled to 80 K, and illuminated the protein is trapped in its K state. A shift of 2 $cm^{-1}$ between SRII (3626 $cm^{-1}$) and SRII-HtrII (3628 $cm^{-1}$) is found for a negative OH stretching band assigned to an internal water molecule, most likely located near the active Schiff base. In contrast, the OH stretching band for this water in the K state appears at the same frequency (3619 $cm^{-1}$) for both the free and complexed receptor. Similar shifts are observed upon hydration with $H_2O^{18}$ shifted to a lower frequency confirming these bands arise from the OH stretching mode of water. Data are also presented on the effects of lipid environment on structural changes of internal water molecules and the receptor-transducer interactions. This work was supported by National Institutes of Health Grants
R01GM069969 (to K.J.R.) and R37GM27750 (to J.L.S.), U.S. Department of Energy Grant DE-FG02-07ER15867, and the Robert A. Welch Foundation (to J.L.S.).

**1503-Pos**
**Exploring the Mechanics and Energetics of Epidermal Growth Factor Receptor Activation**
**Marco Cavalli**, Marco Ceruso.
The City College of New York, New York, NY, USA.
The activation of epidermal growth factor receptor (EGFR) is a complex molecular process. To date, because of technical limitations in dealing with a full-length receptor construct, our understanding of this process comes from structural and biochemical studies of isolated fragments of the receptor. Here we seek detailed molecular insight into the activation process of EGFR in the context a full-length receptor construct. To be able to handle computationally such a large protein (~1000 amino acids) we have developed ELNEDIN: a coarse-grained modeling approach that can describe reliably the dynamics and interactions of proteins. We have built full-length models of human EGFR in the active (extended) and inactive (tethered) states. The models include explicit representations of the lipid membrane and aqueous environments. The conformational space of the tethered and extended state at equilibrium was sampled extensively in the microsecond time scale using a combination of classical molecular dynamics and enhanced sampling techniques. Using these enhanced sampling techniques we have also generated paths that describe the transition from the tethered to the extended state, i.e. the activation of the receptor. These paths have yielded a clear molecular picture of the sequence of conformational changes that lead to activation of EGFR. This picture is remarkably consistent with that derived from experimental approaches, but it also provides new insights into the activation process. Notably, it shows how the conformational changes that occur on the extracellular side of the membrane affect the structure dynamics of the intracellular components of the receptor. Finally, the free-energy surface associated the activation was obtained